Developing a Guided Procedure for Troubleshooting HPLC and UHPLC Systems

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Overview

Purpose: Develop a robust, user-friendly, and fast generic approach for troubleshooting pharmaceutical HPLC and UHPLC assays.

Methods: Isocratic and gradient test mixtures are separated on a reference column for assessing performance of instrument and separation column.

Results: The chromatographic performance is evaluated by an application independent separation. Chromatographic performance indicators are compared with reference values and troubleshooting hints and recommendations are given automatically by using the capabilities of the Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software.

Introduction

During sample analysis and interpretation things can go wrong. Problems can occur strarting with sampling and sample preparation. Also during the actual analysis and reporting things can go wrong and might require corrective action. For methodical troubleshooting it is important to follow a systematic approach for finding the origin of the observed deviation from the expected performance. The presented work focuses on a troubleshooting solution which can easily differentiate between instrument or chemistry (i.e. column or mobile phase) issues. The concept of our new methodical troubleshooting solution is to identify key parameters of chromatographic performance using standardized test mixes run with standardized reference methods and a selected reference column. The test methods are uploaded as electronic workflows providing a completely setup system within a few mouse clicks. A fully automated and preprogrammed custom report offers immediate interpretation of the investigated troubleshooting parameters. Electronic workflows and reports are part of the software tools provided with the described troubleshooting solution.

Method

Liquid Chromtaography System

Thermo Scientific™ Dionex™ UltiMate™ 3000 RS system with: degasser SRD-3400, binary pump HPG-3400RS, sampler WPS-3000TRS, column thermostat TCC-3000RS and UV- detector VWD-3400RS.

Thermo Scientific XPert Troubleshooting Solution (PN 3200.0001) consisting of Reference Column (Thermo Scientific™ Accucore™ XL C18, 3 x 100 mm, 4 μm , PN 74104-103030), XPert isocratic test mix , XPert gradient test mix, and eWorkflows™.

Data Analysis

Chromeleon CDS 7.2 with service release package SR1.

Isocratic Test Conditions

Mobile Phase: Water/Acetonitrile (50/50 v/v)
Flow rate: 640 μL/min
Temperature: 30°C
Injection Volume: 1 μL
Detection: 254 nm, 25 Hz data collection rate

Gradient Test Conditions

Mobile Phase: A-Water, B-Acetonitrile
Gradient: 0–3.8 min 40–95% B, 3.8–4.5 min 95% B, 4.5–6.5 min 40 %B
Flow rate: 800 μL/min
Temperature: 40°C
Injection Volume: 1 μL
Detection: 254 nm, 25 Hz data collectiion rate

FIGURE 1. Isocratic reference chromatogram.

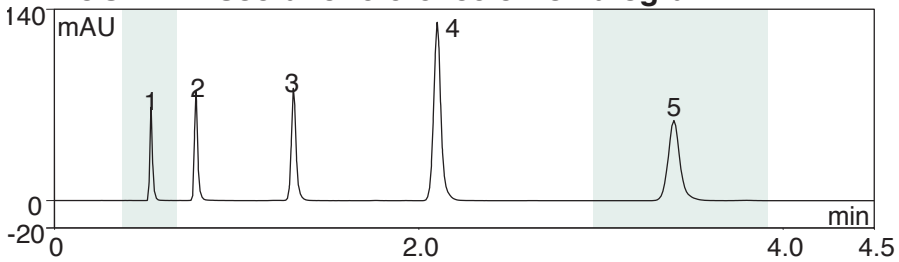
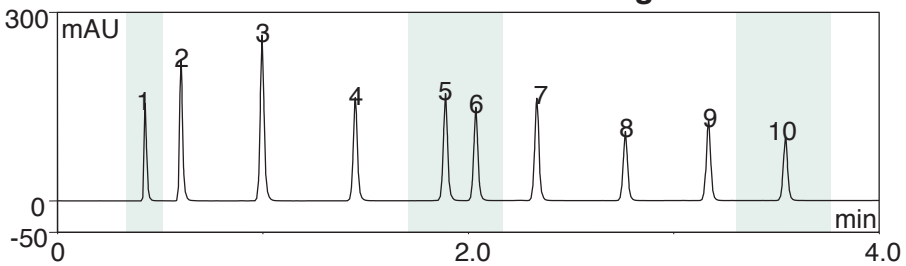


FIGURE 2. Gradient reference chromatogram.



Key Performance Indicators

Chromatographic parameters of the reference separation in isocratic and gradient mode are processed automatically by the CDS. The investigated key indicators for judging the chromatographic performance are listed in table 1. For the isocratic test mode the o-Xylene (peak 5, Figure 1) is investigated. For the gradient test mode the indicators of octanophenone (peak 10 , Figure 2) and butyrophenone and benzophenone as critical peak pair (peak 5/6) are evaluated

TABLE 1. Investigated performance indicators.

Isocratic test (Fig. 1)	Gradient test (Fig. 2)
Retention time window	Retention time window
Retention time RSD	Retention time RSD
Peak asymmetry	Peak asymmetry
Peak area RSD	Peak area RSD
Theoretical plates	Resolution of critical peak pair
Capacity factor	Capacity factor
Generated back pressure	Pressure maximum
Number of peaks	Number of peaks

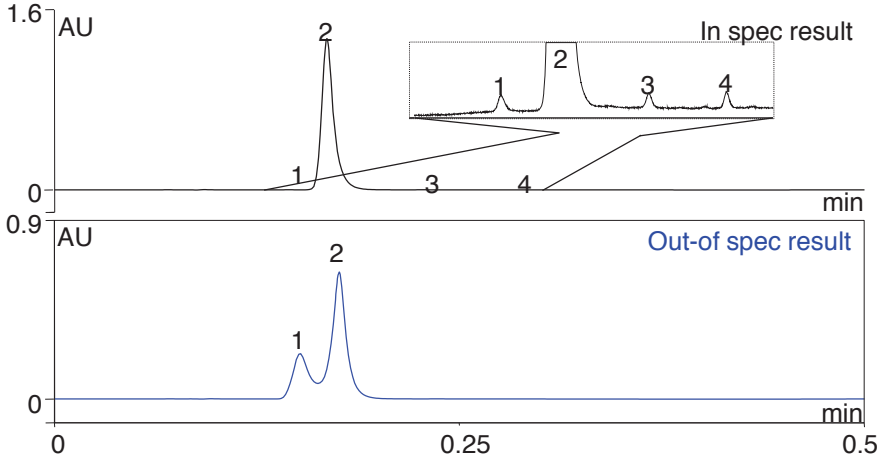
All chromatograms are integrated automatically. The calculated results are checked against specifications values which are embedded in the sequence table. The correct operation of all instrument parts and the characterization of appropriate fluidic connections is evaluated. Finally a final report is generated.

Results

Example: Out-of-Spec Result in Quality Control (QC)

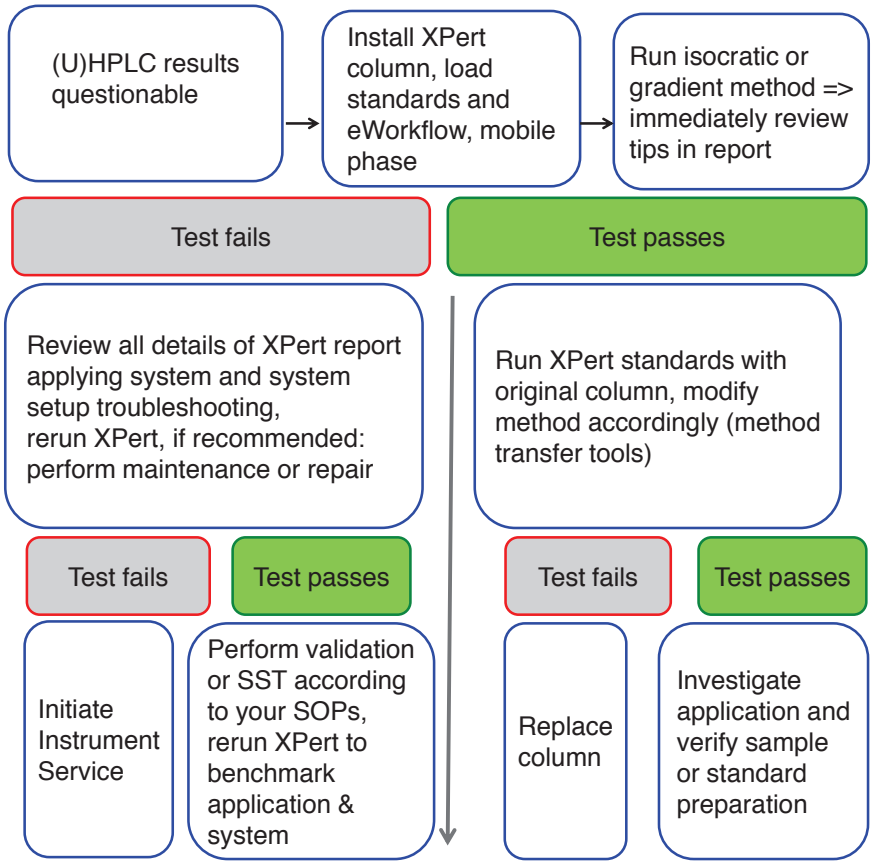
Nevirapine is a non-nucleoside reverse transcriptase-inhibitor with activity against human immunodeficiency virus type 1 (HIV-1). The impurity analysis (see Figure 3) separates the active pharmaceutical ingredient (API, 2) from its impurities A (3), B(1) and C(4). The trendplot of an impurity control of a Nevirapine formulation shows out-of spec results related to the area of one impurity. The outlier result shows an relative area of the 25% for impurity B (Figure 3, Peak 1, bottom) compared to the in spec result of 0.03%.

FIGURE 3. Out-of-spec result in impurity analysis.



The interpretation of the result is performed according to the methodical troubleshooting workflow of Fig. 4.

FIGURE 4. Troubleshooting workflow.



Step I – System Check: First the isocratic test mix is separated on the reference column by easy method installation using the eWorkflow. The results are immediately assed by an report, showing that the system is in an operable status(Fig. 5)

FIGURE 5. Automatically evaluated parameters show operable system.

XPert Performance Test Results

Injection Details

Injection Name:	**XPert RP gradient test mix**	*Run Time (min):*	**6,50**
Vial Number:	**RA3**	*Injection Volume:*	**1,00**
Injection Type:	**Check Standard**	*Channel:*	**UV_VIS_1**
Injection Counter:	**161**	*Wavelength:*	**245,0**
Instrument Method:	**XPert_RP_RS_HPG_VWD**	*Bandwidth:*	**n.a.**
Processing Method:	**Accucore_XL_C18_100x3_gradient_HPG_RS**	*Dilution Factor:*	**1,0000**
Injection Date/Time:	**07.Okt.13 14:17**	*Sample Weight:*	**1,0000**

Xpert Performance Test Result

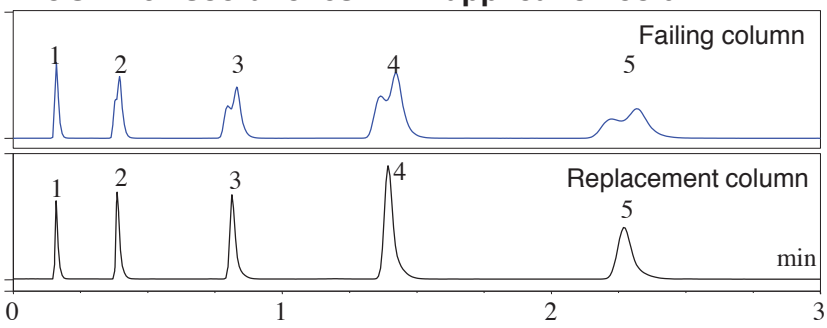
No.	Name	Ref. Value 1	Ref. Value 2	Test Result	Eval. Result
1	Pump Pressure Stability	127,600	n.a.	Passed	117,030
2	Peak Number Total	10,000	n.a.	Passed	10,000
3	Retention Time	3,000	4,000	Passed	3,543
4	RSD of Peak Retention T	0,100	n.a.	Passed	0,022
5	Resolution (USP)	3,000	n.a.	Passed	4,016
6	Peak Asymmetry	0,950	1,200	Passed	1,019
7	RSD of Peak Areas_1	0,500	n.a.	Passed	0,340
8	Peak Width (50%)	0,500	n.a.	Passed	0,022
9	Capacity Factor	6,000	8,000	Passed	7,298

Number of executed test cases: 9 **Total Result:** **Passed**

Step II – Column Check: After checking the system operability the application column performance is evaluated by running the troubleshooting methods. The methods are transferred from the reference column dimension to the application column dimension by using the method transfer tool which is part of the Chromeleon CDS.

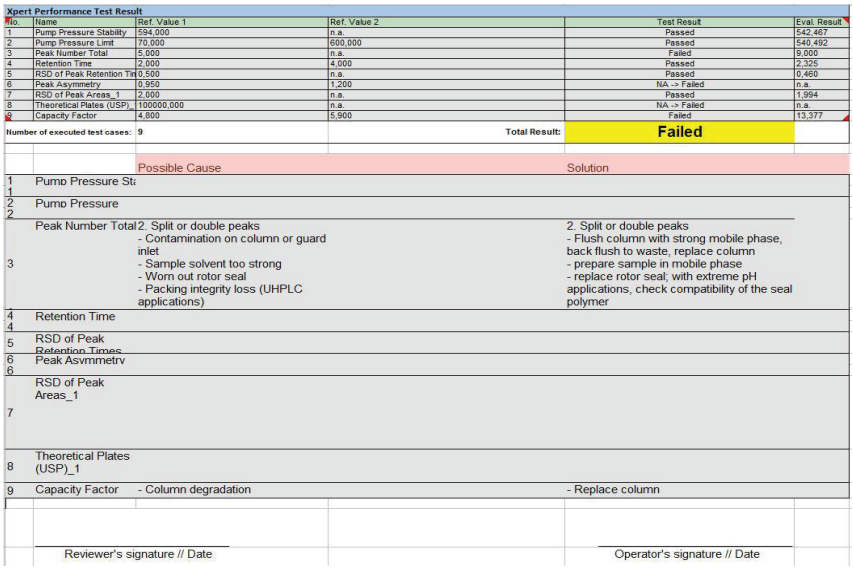
The resulting chromatogram of the isocratic test mix separation is shown in Fig. 6 (blue).

FIGURE 6. Isocratic test with application column.



The specification of “Peak number total” is not fulfilled and immediately a failed test report is given announcing the column failure as a cause and giving the recommendation to replace the column (Fig.7).

FIGURE 7. Failed test result due to specification mismatch.



Xpert Performance Test Result

No.	Name	Ref. Value 1	Ref. Value 2	Test Result	Eval. Result
1	Pump Pressure Stability	594,000	n.a.	Passed	542,467
2	Pump Pressure Limit	70,000	600,000	Passed	540,492
3	Peak Number Total	5,000	n.a.	Failed	9,000
4	Retention Time	2,000	4,000	Passed	2,325
5	RSD of Peak Retention Tin	0,500	n.a.	Passed	0,460
6	Peak Asymmetry	0,950	1,200	NA -> Failed	n.a.
7	RSD of Peak Areas_1	2,000	n.a.	Passed	1,994
8	Theoretical Plates (USP)_	100000,000	n.a.	NA -> Failed	n.a.
9	Capacity Factor	4,800	5,900	Failed	13,377

Number of executed test cases: 9 — Total Result: **Failed**

		Possible Cause	Solution
1	Pump Pressure St		
2	Pump Pressure		
3	Peak Number Total	2. Split or double peaks - Contamination on column or guard inlet - Sample solvent too strong - Worn out rotor seal - Packing integrity loss (UHPLC applications)	2. Split or double peaks - Flush column with strong mobile phase, back flush to waste, replace column - prepare sample in mobile phase - replace rotor seal; with extreme pH applications, check compatibility of the seal polymer
4	Retention Time		
5	RSD of Peak Retention Times		
6	Peak Asymmetry		
7	RSD of Peak Areas_1		
8	Theoretical Plates (USP)_1		
9	Capacity Factor	- Column degradation	- Replace column

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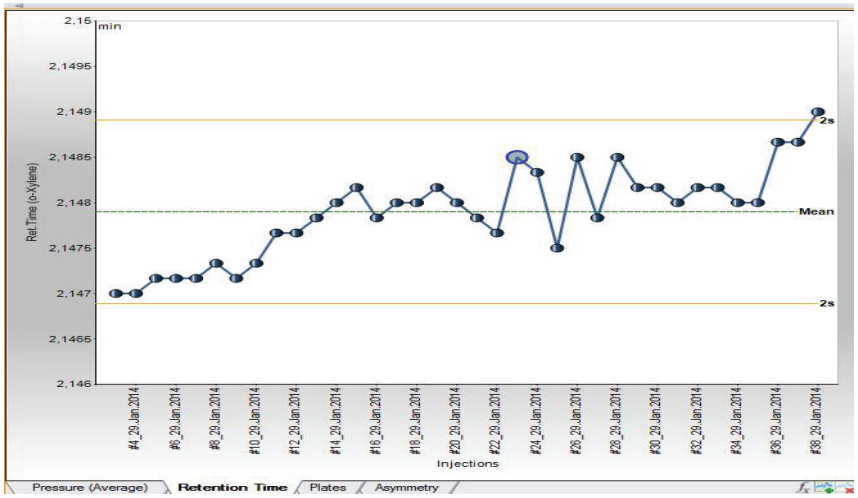
Step III – Replacing the Column

After replacing the column rerunning the isocratic test mix, the operability of the column is given again (Fig. 6, black). The interpretation of the out-of spec result of the Nevirapine impurity can be assessed as column failure.

Step IV – Performance Monitoring

Long term monitoring of chromatographic performance indicators is implemented in the routine workflow by periodically running the test methods on the separation column allowing an early intervention. The visualization is done without exporting by just using the predefined data view settings of the XPert Troubleshooting Solution.

FIGURE 8. Using Chromeleon CDS 7.2 trendplot functionality for performance monitoring.



Conclusion and Outlook

- A standardized check run consisting of reference column and reference test mixture generates all fundamental chromatographic parameters required for system and column troubleshooting independent from the originally used method.
- Automatically evaluated data with instant customized report provide helpful indication on the source of problems and suggested actions to remedy them. Troubleshooting is simplified for LC beginners and allows building own knowledge and practical troubleshooting competence, while making the user aware of key performance indicators and introducing performance monitoring for an early intervention if things go wrong.
- The XPert troubleshooting solution is the next generation of automated troubleshooting and performance monitoring and builds on the high degree of functionality of the Chromeleon 7.2 CDS software.

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